(OP 158) *In Vivo* Engraftment Potential of Human Bone Marrow and Amniotic Fluid Stem Cells Cultured Under Osteogenic Conditions

A.M. Frias[1,2], I.R. Dias[1,2,3], C.A.A. Viegas[1,2,3], S. Fernandes[4], A. Barros[4], J.T. Azevedo[5], N.M. Neves[1,2], R.L. Reis[1,2]

[1]3B's Research Group–Biomaterials, Biodegradables and Biomimetics, Dept. of Polymer Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[2]IBB–Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Braga, Portugal
[3]Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[4]Genetics Department, Faculty of Medicine, University of Porto, Porto, Portugal
[5]CECAV—Centre for Animal Science and Veterinary, Department of Animal Sciences, University of Trás os-Montes and Alto Douro, Vila Real, Portugal

The potential immune privilege presented by mesenchymal stem cells (MSCs) makes them a promising population in tissue engineering and regenerative medicine. In this study, we investigated the effect of culture medium (expansion versus osteogenic media) over the *in vivo* potential of adult stem cells derived from human bone marrow (hBMMSCs) and amniotic fluid (hAFSCs).To this end, we isolated hAFSCs from day 6 supernatant of the cultures of amniotic fluid obtained from amniocentesis, and hBMMSCs were a kind gift from Biopredic. Cells were maintained in culture until confluence either in expansion or in osteogenic media (7 days), and then transplanted into 58 to 62 day-old foetal sheep at a concentration of 1×10^6 cells/foetus. Pregnant ewes were fasted for 24 hours. General anaesthesia was induced with thiopental sodium and maintained by inhalation anaesthesia with isoflurane and oxygen. After general anaesthesia, the ewes were positioned in dorsal recumbency and prepared in a sterile surgical environment for a ventral midline celiotomy. The abdomen was exposed through a ventral midline incision and the gravid uterus located. After the identification of the foetus inside the uterus and their gentle handling contention against the inner epithelium layer of the uterus, cells were transplanted into the intraperitoneal foetus cavity by injection through the intact uterus walls. The animals were euthanized sixty days after transplant, and samples from various tissues were collected. The engraftment and phenotype of human-derived cells was evaluated by flow cytometry and immunocytochemistry analysis.